



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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DATE August 14, 1992

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT 2-ETHYLHEXYL ESTER of 2,4-D ACID: Developmental Toxicity
Studies in Rats and Rabbits As Required in the 2,4-D
Registration Standard.

FROM: Jess Rowland, Toxicologist *Jess Rowland 8/14/92*
Section II, Toxicology Branch II
Health Effects Division (H7509C)

TO: W. Waldrop/J. Coombs
Product Manager (71)
Reregistration Division

THRU: K. Clark Swentzel, Section Head *K. Clark Swentzel 8/19/92*
Section II, Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief *M van Gemert 8/23/92*
Toxicology Branch II
Health Effects Division (H7509C)

PROJECT IDENTIFICATIONS: Submission: 8420266 DP Barcode: D179762

PC Code: 030063 Caswell No. 315 AS

MRID No(s): 423046-01--Main Study--Rats
423046-02--Range Finding Study--Rats
423046-03--Main Study--Rabbits
423046-04--Range Finding Study--Rabbits

Registrant: Industry Task Force II on 2,4-D Research Data.

ACTION REQUESTED: Review of developmental toxicity and probe
studies in rats and rabbits with the 2-Ethylhexyl ester [Isooctyl
Ester] of 2,4-Dichlorophenoxyacetic Acid.

RESPONSE: A Data Evaluation Report [DER] for each of the above
referenced studies is attached. A summary of the DER is provided
below:

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1. **DOSAGE-RANGE DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCYL ESTER] ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD BR VAF/Plus PRESUMED PREGNANT RATS [MRID No. 423046-02].**

SUMMARY: In a dose-range finding study, pregnant rats were given oral administration of technical 2,4-D-2-ethylhexyl ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 50, 100, 150 or 200 mg/kg/day during days 6 through 15 of gestation. Does were sacrificed on gestation day 20. No maternal toxicity was seen at 50 mg/kg/day. At 100, 150 and 200 mg/kg/day 2,4-D-2-EHE induced maternal toxicity was evident from mortality/morbidity, clinical signs of toxicity, decreases in body weight gain and food consumption, and gross pathological alterations. Treatment had no adverse effects on pregnancy rate, the number of implantations, the number of resorptions, litter size, or viability of fetuses at 50 or 100 mg/kg/day. Treatment-related effects seen at 100, 150 and/or 200 mg/kg/day included: a reduction in live litter sizes; increase in the mean number of resorptions per litter; increase in the percentage of resorbed fetuses per litter; and increases in the numbers of totally resorbed fetuses.

MATERNAL TOXICITY NOEL = 50 mg/kg/day [LDT]; LOEL = 100 mg/kg/day

DEVELOPMENTAL TOXICITY NOEL = 100 mg/kg/day; LOEL = 150 mg/kg/day

CORE CLASSIFICATION: Not applicable; range-finding study.

2. **DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD BR VAF/Plus PRESUMED PREGNANT RATS [MRID No. 423046-01].**

SUMMARY: Groups of 20 pregnant rats were given oral administration of technical 2,4-D-2-ethylhexyl ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 10, 30, or 90 mg/kg/day during days 6 through 15 of gestation. Does were sacrificed on Day 20. No maternal toxicity was seen at 10 mg/kg/day. At 30 mg/kg/day, maternal toxicity was limited to decreases in body weight gain and the presence of a red perivaginal substance which probably reflected reduced grooming. At 90 mg/kg/day, maternal toxicity included clinical signs of toxicity, and decreases in body weight gain and food consumption. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external or visceral malformation or variations were seen in any of the fetuses of treated does. A skeletal variation attributable to treatment was the delayed sternal ossification [incomplete and unossified sternebrae] observed at 30 and 90 mg/kg/day. Based on the results of this study, the following NOELs and LOELs are established:

MATERNAL TOXICITY NOEL = 10 mg/kg/day; LOEL = 30 mg/kg/day

DEVELOPMENTAL TOXICITY NOEL = 10 mg/kg/day; LOEL = 30 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rats (83-3) and is acceptable for regulatory purposes.

3. **DOSAGE-RANGE DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY [STOMACH TUBE] TO NEW ZEALAND WHITE RABBITS [MRID No. 423046-04].**

SUMMARY: In a dose range-finding study, impregnated New Zealand White rabbits were given oral administration of technical 2,4-D-2-Ethylhexyl Ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 50, 100, 150 or 200 mg/kg/day during days 6 through 18 of gestation. Does were sacrificed on gestation day 29. No maternal toxicity was seen at 50 mg/kg/day. At 100, 150 and 200 mg/kg/day, 2,4-D-2-EHE induced maternal toxicity which included mortality/morbidity, clinical signs of toxicity, decreases in body weight gain and food consumption, and gross pathological alterations. Treatment had no adverse effects on pregnancy rate, the number of implantations, the number of resorptions, litter size, or viability of fetuses at 50 mg/kg/day. However, maternal toxicity at higher dose levels precluded meaningful evaluation of these parameters at higher levels. No fetal data were reported.

MATERNAL TOXICITY NOEL = 50 mg/kg/day [LDT]; LOEL = 100 mg/kg/day

DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day

CORE CLASSIFICATION: Not applicable; range-finding study.

4. **DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY [STOMACH TUBE] TO NEW ZEALAND WHITE RABBITS [MRID No. 423046-03].**

SUMMARY: Groups of 20 inseminated rabbits were given oral administration of technical 2-ethylhexyl ester of 2,4-D [63.25% acid equivalent, 95% pure,] at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 18 of gestation. 2,4-D-2-EHE at 10 or 30 mg/kg/day did not induce maternal toxicity. At 75 mg/kg/day maternal toxicity was manifested by mortality/morbidity, decreases in mean body weight, body weight gain and food consumption in does that died or were sacrificed moribund. Surviving does exhibited clinical signs of toxicity that included dried feces, decreased motor activity, ataxia, impaired righting reflex, lost righting reflex and bradypnea. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external, visceral, or skeletal malformation or variations were seen in any of the fetuses of treated does. Based on these results the following NOELs and LOELs are established.

MATERNAL TOXICITY: NOEL = 30 mg/kg/day; LOEL = 75 mg/kg/day [HDT]

DEVELOPMENTAL TOXICITY: NOEL = 75 mg/kg/day; LOEL= Not Achieved

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

PRIMARY REVIEWER: Jess Rowland, Toxicologist *Jess Rowland 8/14/92*
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head *K. Clark Swentzel 7/19/92*
Section II, Toxicology Branch II

DATA EVALUATION REPORT

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1. RANGE-FINDING STUDY--RATS

STUDY TYPE: Developmental Toxicity [Range-Finding] **GUIDELINE:** N/A

CASWELL NO. 315 AS **MRID No.** 423046-02 **DP Barcode:** D179762

TEST MATERIAL: 2-Ethylhexyl Ester of 2,4-D ACID [2,4-D-EHE]

REGISTRANT: Industry Task Force on 2,4-D Research Data.

TESTING LABORATORY: Argus Research Laboratories, PA

STUDY IDENTIFICATION: Argus 320-005P

TITLE OF REPORT: DOSAGE-RANGE DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD BR VAF/Plus PRESUMED PREGNANT RATS.

AUTHOR: Terry Martin, D.V.M **REPORT DATE:** April 10, 1992

SUMMARY: In a dose-range finding study, pregnant rats were given oral administration of technical 2,4-D-2-ethylhexyl ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 50, 100, 150 or 200 mg/kg/day during days 6 through 15 of gestation. Does were sacrificed on gestation day 20. No maternal toxicity was seen at 50 mg/kg/day. At 100, 150 and 200 mg/kg/day, 2,4-D-2-EHE induced maternal toxicity was evident from mortality/ morbidity, clinical signs of toxicity, decreases in body weight gain and food consumption, and gross pathological alterations. Treatment had no adverse effects on pregnancy rate, the number of implantations, the number of resorptions, litter size, or viability of fetuses at 50 or 100 mg/kg/day. Treatment-related effects seen at 100, 150 and/or 200 mg/kg/day included: a reduction in live litter sizes; increase in the mean number of resorptions per litter; increase in the percentage of resorbed fetuses per litter; and increases in the numbers of totally resorbed fetuses.

MATERNAL TOXICITY NOEL = 50 mg/kg/day [LDT]; **LOEL** = 100 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; **LOEL** = 100 mg/kg/day

CORE CLASSIFICATION: Not applicable; range-finding study.

1. **OBJECTIVE**

The objective of this range-finding study in rats was to establish appropriate dose levels of the 2-ethylhexyl ester of 2,4-D [2,4-D-2-EHE] for the main study.

2. **PROTOCOL**

Groups of eight presumed pregnant Crl:CD BR VAF/Plus [Sprague-Dawley] rats [approximately 1 month of age and weighing 200 to 225 g] were given oral administrations of Technical 2,4-D-2-EHE [63.25% acid equivalent, 95% pure, Lot No.04KF54479] at acid equivalent doses of 50, 100, 150, or 200 mg/kg/day in 1% methylcellulose [10 mL/kg] daily, during days 6 through 15 of gestation. A control group received the vehicle alone under the same schedule. Concentration and homogeneity of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed daily for viability, clinical signs of toxicity, abortions, premature deliveries, body weights and food consumption during the dosing and post-dosing periods. Dams in each group were sacrificed on day 20 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. Fetal examinations were not performed.

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 144%, 82%, 94% and 103% of the nominal concentration, respectively, on the first day of dosing, and 109%, 122%, 89%, and 74%, respectively, on the last day of dosing for the 50, 100 and 150 mg/kg/day groups. Dosing solution exhibited 100% homogeneity.

ii. Maternal Toxicity

- o 50 mg/kg/day: No mortality or moribundity.
- o 100 mg/kg/day: 1 dam found dead on Day 18.
- o 150 mg/kg/day: No mortality or moribundity.
- o 200 mg/kg/day: 1 doe each was found dead on Days 11 & 14 and 1 dam was sacrificed moribund on Day 14.
- o No clinical signs of toxicity were seen at 50 mg/kg/day. Treatment-related clinical signs observed in surviving dams included ataxia, decreased motor activity, pinpoint pupils [100, 150 and 200 mg/kg/day]; impaired righting reflex [150 and 200 mg/kg/day]; and urine stains on the fur and red vaginal substance [200 mg/kg/day]. The severity and incidence of these signs generally remained constant throughout the course of the dosing period.
- o When compared to controls, the mean body weight gain was unaffected at 50 mg/kg/day. Decreases in average weight gain were seen at 100, 150 and 200 mg/kg/day during the dosing-[Days 6 to 16] and post-dosing [Days 16 to 20] periods. Reduction in the corrected body weight gain was noted at 150 and 200 mg/kg/day. Average gravid uterine weights was reduced at 150 and 200 mg/kg/day.
- o Food consumption was unaffected at 50 mg/kg/day. When compared to controls, decreases in absolute [g/day] and relative [g/kg/day] food consumption occurred during the entire dosing period at 100 mg/kg/day and higher dosage groups. During the post-dosing period, absolute food consumption was slightly reduced at 150 and 200 mg/kg/day.
- o Treatment-related gross pathological alterations seen only in the rat that was found dead at 100 mg/kg/day were gastric erosions and a bright to dark red lungs. In rats that died or sacrificed at 200 mg/kg/day, necropsy revealed gastric erosions, bright to dark red lungs, consolidated lungs and a yellow to red mottled liver.

iii. Developmental Toxicity

- o The pregnancy rate was 100% in all treatment groups.
- o No marked treatment-related effects were observed in implantations, litter sizes, or viable fetuses at 50 mg/kg/day when compared to controls.
- o The increase observed in the average number of resorptions [1.6 ± 0.8] when compared to controls [1.1 ± 1.1] was within the historical control range [0.3 to 2.0] of the testing laboratory.
- o The increases in the number of dams with any resorptions at 100, 150 and 200 mg/kg/day was higher than the concurrent controls, and were outside the historical control range [25 to 82%]. Therefore, this finding was considered to be treatment related.
- o Treatment-related effects observed in caesarean sectioning at 150 and 200 mg/kg/day are tabulated below:

C-Section Observations	mg/kg/day				
	0	50	100	150	200
Litter size [mean]	13	15	13	11	5
Total No. of live fetuses	102	116	90	85	27
Resorptions [mean]	1.1	0.6	1.6	4.4	9.4
Early Resorptions [mean]	1.1	0.6	1.6	4.4	9.2
Dams with any resorptions	63%	38%	100%	88%	100%
Mean percentage of resorbed conceptuses/Litter	10	4	11	17	41

- o No data were reported for fetal examinations.

4. CONCLUSION:

MATERNAL TOXICITY: NOEL = 50 mg/kg/day [LDT]; LOEL = 100 mg/kg/day

DEVELOPMENTAL TOXICITY: NOEL = 50 mg/kg/day; LOEL = 100 mg/kg/day

5. CORE CLASSIFICATION: Not applicable; range-finding study.

PRIMARY REVIEWER: Jess Rowland, Toxicologist *Jess Rowland 8/14/92*
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head *K. Clark Swentzel*
Section II, Toxicology Branch II *8/19/92*

DATA EVALUATION REPORT

2. MAIN STUDY--RATS

STUDY TYPE: Developmental Toxicity [Main Study] **GUIDELINE:** 83-3(b)

CASWELL NO. 315 AS **MRID No.** 423036-01 **DP Barcode:** 179762

TEST MATERIAL: 2-Ethylhexyl Ester of 2,4-D [2,4-D-EHE]

REGISTRANT: Industry Task Force II on 2,4-D Research Data

STUDY IDENTIFICATION: Argus 320-005

TESTING LABORATORY: Argus Research Laboratories, PA.

TITLE OF REPORT: DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD BR VAF/Plus PRESUMED PREGNANT RATS.

AUTHOR: Terry Martin, D.V.M **REPORT DATE:** April 10, 1992

SUMMARY: Groups of 20 pregnant rats were given oral administration of technical 2,4-D-2-ethylhexyl ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 10, 30, or 90 mg/kg/day during days 6 through 15 of gestation. Does were sacrificed on Day 20. No maternal toxicity was seen at 10 mg/kg/day. At 30 mg/kg/day, maternal toxicity was limited to decreases in body weight gain and the presence of a red perivaginal substance which probably reflected reduced grooming. At 90 mg/kg/day maternal toxicity included clinical signs of toxicity, and decreases in body weight gain and food consumption. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external or visceral malformation or variations were seen in any of the fetuses of treated does. A skeletal variation attributable to treatment was the delayed sternal ossification observed at 30 and 90 mg/kg/day. Based on the results of this study, the following NOELs and LOELs are established:

MATERNAL TOXICITY NOEL = 10 mg/kg/day; LOEL = 30 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 10 mg/kg/day; LOEL = 30 mg/kg/day.

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rats (83-3) and is acceptable for regulatory purposes.

I. OBJECTIVE

The objective of this study was to assess the effects of the 2-Ethylhexyl ester of 2,4-D [2,4-D-EHE] on the embryonic and fetal development following oral administration to rats during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: 2-ethylhexyl ester of 2,4-D [Technical]
Batch No.: 04KF54479
Acid Equivalent: 63.25%
Purity: 95%
Description: Yellow liquid

b. Test Animals

Species/Sex: Female rats
Strain: Crl:CD BR VAF/Plus
Age at receipt: Approximately 65 days
Weight on Day 0: 242 to 292 g
Identification: Ear tags.
Acclimation Period: Approximately 2 weeks.
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5002 ad libitum.
Water: Tap water ad libitum
Environment: Temperature-70-78°F; humidity-40-70%; light cycle:12 hr. light/12 hr.dark.

Group Assignment: 25 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Following an acclimation period, virgin females were placed in cohabitation with males in a 1:1 ratio. Female rats with spermatozoa observed in smears of vaginal contents and/or copulatory plugs observed in situ were considered to be at day 0 of presumed gestation and assigned to individual housing.

d. Preparation of Dosing Solutions

Suspensions of 2,4-D-EHE in aqueous 1% methylcellulose were prepared daily. Dosage calculations were corrected for the 63.25% acid equivalence of the test substance and expressed as mg of the acid.

e. Analysis of the Dosing Solutions

Concentration analysis of the dosing solutions were determined twice [on the first and the last days] during the study. Homogeneity analyses of the mid and high dosage concentrations were performed prior to the beginning of the dosage period, and the low dose was analyzed for homogeneity on the second day of dosing. Since dosing samples were prepared daily, no additional stability analyses were performed.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 15 of gestation. All groups received a dosing volume of 10 mL/kg body weight and the dose volumes were adjusted daily based on individual body weights. Each daily dosage given to the rats was administered at approximately the same time each day.

g. Observations

All animals were observed daily during the dosing and post-dosing periods for clinical signs of toxicity, abortions, premature deliveries and deaths. Individual body weights were obtained on day 0 and daily during the dosing and post-dosing period. Individual food consumptions were measured daily during the study period.

h. Termination

Any animal which died, appeared moribund or showed indications of early termination of pregnancy was submitted for complete necropsy. All surviving dams were sacrificed on gestation day 20, obvious gross pathologic alterations were recorded, and the gravid uteri were weighed.

i. Cesarean Section

The thoracic, abdominal and pelvic cavities were examined for gross lesions, and in the event of gross lesions, the tissues were preserved in neutral buffered 10% formalin. The uterus was removed from the body, examined externally, weighed and then opened for internal examination. Uteri that appeared to be from nonpregnant rats were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantations, early and late resorptions, and live and dead fetuses were recorded.

j. Fetal Examinations

Each fetus was removed from the uterus and individually weighed, and observed for gross external alterations. Every fetus was examined to determine sex and soft tissue alterations. Fetuses were then eviscerated, stained with Alizarin red-S, and examined for skeletal alterations.

k. Statistical Analysis

Maternal body weights, body weight gains, gravid uterine weights, feed consumption data, and litter averages for fetal body weights, percent male fetuses, percent resorbed conceptuses, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance [ANOVA]. If the ANOVA was significant, analysis by Dunnett's Test was used. If the ANOVA was not significant, the Kruskal-Wallis Test or Fisher's Exact Test was used. All other cesarean-sectioning data were evaluated using the Kruskal-Wallis Test. Observations for aborted conceptuses and late resorptions were excluded from statistical analyses.

l. Compliance Statements:

A signed Statement of No Confidentiality Claim was provided and dated April 10, 1992.

A signed Statement of Compliance with EPA's GLP was provided that was dated April 10, 1992.

A signed Quality Assurance Statement was dated April 10, 1992. This date conforms to the review of the study phases and the draft and the final reports.

A signed statement dated April 10, 1992 was provided, which indicated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the study. This study neither reportedly met or exceeded any of these criteria.

III. RESULTS

Analysis of the Dosing Solutions

Results of the concentration analyses of the dosing solutions are tabulated below:

Target Level		% Deviation from Target Dosage	
mg/mL	mg/kg/day	First Day Sample	Last Day Sample
1.0	10	4	-36
3.0	30	-1	-4
7.5	90	-4	-4

The -36% deviation from the target dose level for the low-dose on the last day of dosing was not considered to compromise the study because: (i) it represented a single preparation day; (ii) the concentration analyses of the 10 mg/kg/day dosing solution in the rabbit study, which used the same mixing procedures, yield a -7% deviation from the target level; and (iii) the analytical precision data collected on the third day of dosing at the 10 mg/kg/day level showed the concentration to be within $\pm 10\%$.

Results of the homogeneity analyses showed a relative standard deviation of 8% for the 1.0 mg/mL level, 4% for the 5.0 mg/mL level, and 5% for 20.0 mg/mL.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

No maternal mortality/morbidity, abortions or premature delivery occurred at any dose level.

b. Clinical Signs

No clinical signs of toxicity were seen at 10 mg/kg/day. Statistically significant, treatment-related clinical signs of toxicity observed were: the presence of a red perivaginal substance generally during Days 13, 14, or 15 of gestation in 8 dams at 30 mg/kg/day [$p < 0.05$] and in 12 dams at 90 mg/kg/day [$p < 0.1$]; ataxia in 4 dams at 90 mg/kg/day [$p < 0.1$]; and decreased motor activity and bradypnea in 1 dam at 90 mg/kg/day.

c. Mean Body Weight and Body Weight Gains

No treatment-related effects were seen in mean body weights at any dose level. Body weight gain was unaffected at 10 mg/kg/day. However, statistically significant [$p < 0.05$ to $p < 0.01$] differences in body weight gains were seen during the early dosing period [Days 6 to 9] at 30 and 90 mg/kg/day and for the entire dosing period [Days 6-16] at 90 mg/kg/day. When the body weights were corrected for the gravid uterine weights, body weight gains were comparable between the treated and the control group.

Dose mg/kg/day	Mean Body weight Gain [G]			
	Days 0 - 6	Days 6 - 9	Days 6 - 16	Days 6 - 20
0	+37.2±5.6	+18±5.0	+76.2±9.4	+60.9±11.9
10	+37.2±7.3	+13.9±4.8	+77.3±12.0	+61.8±12.7
30	+36.1±6.7	+13.3±4.2**	+71.3±10.8	+57.9±9.6
90	+35.2±7.8	+13.3±7.0*	+66.8±13.1**	+55.7±12.4

d. Food Consumption

No treatment-related or statistically significant differences were observed in absolute [g/day] or relative [g/kg/day] food consumption values at 10, or 30 mg/kg/day. At 90 mg/kg/day, both the absolute and relative food consumption values were significantly [$p < 0.01$] reduced for the entire dosing period.

e. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination.

2. Developmental Toxicity

Reproduction data are presented in Table 1. No biologically or statistically significant effects were seen on pregnancy rate, number of implantations, total live fetuses per litter, resorption rate, number and percent of litters with resorptions, fetal sex ratio, or gravid uterine weights at any dose level. The incidences were reported to be within the historical control ranges of the testing laboratory. This was substantiated with historical control data from the testing laboratory. Fetal body weight was significantly [$p < 0.05$] reduced at 90 mg/kg/day.

Table 1. Cesarean Section Observations

Observations [Mean \pm S.D.]	Dose Level [mg/kg/day]			
	0	10	30	90
No. Assigned	25	25	25	25
Females Gravid	22	22	24	24
<u>Maternal Wastage</u>				
# Died	0	0	0	0
# Sacrificed	0	0	0	0
# Aborted	0	0	0	0
# Early delivery	0	0	0	0
# Non pregnant	3	3	1	1
Total Corpora Lutea	432	431	504	456
Corpora Lutea/Dam	19.6 \pm 3.5	19.6 \pm 3.5	19.1 \pm 3.0	19.0 \pm 2.3
Total Implantations	385	381	411	421
Implantation/Dam	17.5 \pm 2.7	17.3 \pm 3.8	17.1 \pm 3.8	17.5 \pm 1.7
Total Live Fetuses	351	366	378	400
Live Fetuses/Litter	16.0 \pm 3.2	16.6 \pm 3.9	15.8 \pm 3.7	16.7 \pm 2.0
Total Resorptions	34	15	33	21
Early	33	14	33	21
Late	1	1	0	0
Resorption/Dam	1.5 \pm 1.3	0.7 \pm 0.9	1.4 \pm 1.2	0.9 \pm 0.9
No. and % of Litters with Resorptions	16/22 72.7	9/22 40.9	18/25 75.0	14/24 58.3
Pre Implantation Loss [%]	10.9	11.6	18.4	7.7
Post Implantation Loss [%]	8.8	3.9	8.0	5.0
Gravid Uterus Weight [g]	90 \pm 15	95 \pm 21	92 \pm 20	89 \pm 11
Sex Ratio σ / φ	168/183	189/177	173/205	188/212
Fetal Weight[g]/Litter	3.49 \pm 0.2	3.6 \pm 0.2	3.6 \pm 0.4	3.29 \pm 0.3*

As summarized in Table 2, increases in fetuses with any alteration was observed at 30 and 90 mg/kg/day with the increase reaching statistical significance [$p < 0.05$] at the high dose. These occurrences were due to the retarded sternal ossification [see discussion under skeletal examination]. No other statistically or biologically significant differences were seen in fetal alterations at any dose level.

Table 2. Summary of Fetal Alterations

Observations	Dose Level [mg/kg/day]			
	0	10	30	90
Litters Evaluated	22	22	24	24
Fetuses Evaluated	351	366	378	400
Litters with Fetuses with any Alterations Observed	9 40.9%	9 40.9%	15 62.5%	12 50.0%
Fetuses with any Alterations Observed	13 3.7%	12 3.3%	23 6.1%	31 7.8%
Percentage of Fetuses with any Alterations/Litter [Mean±S.D]	3.4± 5.2	4.5±7.8	6.1±6.5	7.9±11.6

The fetal malformations/alterations observed in the 351, 366, 378 and 400 fetuses of does at 0, 10, 30, and 90 mg/kg/day and summarized in Tables 10 thru 13 of the study report [pages 52-58] are appended to this DER.

a. External Examinations

No treatment-related or statistically significant gross external malformations or variations were seen at any dose level.

b. Visceral Examinations

No treatment-related or statistically significant soft tissue malformations or variations were observed.

c. Skeletal Examinations

No statistically significant or treatment-related skeletal alterations were seen at 10 mg/kg/day.

Delayed sternal ossification [i.e., incompletely or unossified sternebrae] occurred in 8, 3, 13 and 18 fetuses in 6, 2, 12 and 9 litters in the 0, 10, 30, and 90 mg/kg/day groups, respectively. At 30 mg/kg/day, the litter incidence [12/24, 50%] was significantly [$p < 0.5$] increased when compared to controls [6/22, 27.3%], and also exceeded the historical control range [0-18.2%] of the testing laboratory. The fetal incidence [13/195, 6.7%] although, was not significantly increased when compared to controls [8/182, 4.4%] did slightly exceed the historical control range [0-5.5%] of the lab.

At 90 mg/kg/day, the fetal incidence [18/207, 8.7%] was significantly [$p < 0.5$] increased when compared to controls [8/182, 4.4%], and also exceeded the historical control range [0-5.5%] of the testing laboratory. The litter incidence [9/24, 37.5%], although was not significantly increased when compared to controls [6/22, 27.3%] did exceed the historical control range [0-18.2%] of the lab. Additionally, the litter average for ossified sternal centers per fetus was significantly reduced at this level; 3.29 ± 0.34 in the treated compared to 3.67 ± 0.34 in the controls.

The delayed sternal ossifications observed at 30 mg/kg/day are considered to be treatment-related since: (i) the litter incidence [the parameter considered more appropriate for analyses] was significant when compared to concurrent controls; (ii) the values were not within the ranges of the historical controls [contrary to what the report author asserts on page 33, paragraph 3]; and (iii) the fetal incidence also slightly exceeded the historical control range. At 90 mg/kg/day, the delayed ossification was associated with reduced fetal body weights.

Other skeletal observations which showed statistical significance [fetal incidence of wavy ribs at 10 and 90 mg/kg/day and incompletely ossified pubes at 30 mg/kg/day] were not considered to be treatment-related since there was no dose-response and the values were within the historical control ranges of the testing laboratory.

IV. DISCUSSION

Pregnant rats were given oral administration of 2-ethylhexyl ester of 2,4-D at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 15 of gestation.

2,4-D-2-EHE at 10 mg/kg/day did not induce maternal toxicity. Treatment did not cause mortality, morbidity, abortions or premature delivery at any dose level. At 30 mg/kg/day, maternal toxicity was limited to decreases in body weight gain and the presence of a red perivaginal substance which probably reflected reduced grooming. At 90 mg/kg/day maternal toxicity was manifested by clinical signs of toxicity that included the presence of a red perivaginal substance, ataxia, decreased motor activity and bradypnea, and decreases in body weight gain and food consumption. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external or visceral malformation or variations were seen in any of the fetuses of treated does. No treatment-related skeletal malformations were seen. Skeletal variation attributable to treatment was the delayed ossification which was seen at 30 and 90 mg/kg/day dose levels. Although, these observations are generally considered reversible delays in ossification they were considered to be treatment related since the values were significant when compared to controls, exceeded the historical control ranges, and was associated with the significantly reduce fetal body weight at the high dose.

In a developmental toxicity study [MRID No. 423046-03] with rabbits, artificially inseminated rabbits were given oral administration of 2,4-D-2-EHE [same lot number and purity (95%) as the rat study] at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 - 18 of gestation. For maternal toxicity the NOEL was 30 mg/kg/day and the LOEL, based on mortality, clinical signs of toxicity and decreases in body weight gain and food consumption was 75 mg/kg/day. For developmental toxicity the NOEL was 75 mg/kg/day, the highest dose tested; a LOEL was not achieved. The lack of any developmental toxicity in rabbits compared to skeletal variations in rats observed in this study at comparable acid equivalent doses [75 mg/kg/day in rabbit vs 90 mg/kg/day in rats] indicate the species difference in 2,4-D-EHE induced developmental toxicity.

V. CONCLUSION

2,4-D-2-EHE was maternally toxic to rats at a dose of 30 and 90 mg/kg/day; no maternal toxicity was seen at 10 mg/kg/day. The compound did not induce developmental toxicity at 10 mg/kg/day, but did cause delays in sternal ossification at 30 and 90 mg/kg/day. Based on the results of this study, the following NOELs and LOELs are established.

Maternal Toxicity

NOEL: 10 mg/kg/day

LOEL: 30 mg/kg/day

Developmental Toxicity:

NOEL: 10 mg/kg/day

LOEL: 30 mg/kg/day

VI. CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rats (83-3 a) and is acceptable for regulatory purposes.

009697

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 10 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	351	366	378	400
Live Fetuses	N	351	366	378	400
Dead Fetuses	N	0	0	0	0

BODY:

Umbilical Hernia

Litter Incidence	N(%)	0	0	1(4.2)	0
Fetal Incidence	N(%)	0	0	1(0.3)	0

a. Dosage occurred on days 6 through 15 of gestation.

009697

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 11 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	169	176	183	193
Live Fetuses	N	169	176	183	193
Dead Fetuses	N	0	0	0	0

NASAL PASSAGES:

Dilated

Litter Incidence	N(2)	0	1(4.5)	0	0
Fetal Incidence	N(2)	0	1(0.6)	0	0

KIDNEYS:

Pelvis. Slight to Moderate Dilation

Litter Incidence	N(2)	2(9.1)	1(4.5)	1(4.2)	1(4.2)
Fetal Incidence	N(2)	2(1.2)	1(0.6)	1(0.5)	1(0.5)

Small

Litter Incidence	N(2)	0	0	1(4.2)	0
Fetal Incidence	N(2)	0	0	1(0.5)	0

a. Dosage occurred on days 6 through 15 of gestation.

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 12 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	182	190	195	207
Live Fetuses	N	182	190	195	207
Dead Fetuses	N	0	0	0	0
VERTEBRAE:					
Thoracic, Centra, Unilateral Ossification					
Litter Incidence	N(X)	1(4.5)	0	0	0
Fetal Incidence	N(X)	1(0.5) ^c	0	0	0
Thoracic, Centra, Not Ossified					
Litter Incidence	N(X)	1(4.5)	0	0	0
Fetal Incidence	N(X)	1(0.5) ^c	0	0	0
Thoracic, Centra, Bifid					
Litter Incidence	N(X)	1(4.5)	1(4.5)	0	0
Fetal Incidence	N(X)	1(0.5) ^c	1(0.5)	0	0
Lumbar, Centrum, Unilateral Ossification					
Litter Incidence	N(X)	1(4.5)	0	0	0
Fetal Incidence	N(X)	1(0.5) ^c	0	0	0
Lumbar, Arches, Incompletely Ossified					
Litter Incidence	N(X)	1(4.5)	2(9.1)	0	2(8.3)
Fetal Incidence	N(X)	1(0.5) ^c	2(1.0) ^{e,f}	0	2(1.0) ^{t,u}
RIB(S):					
Incompletely Ossified (Hypoplastic)					
Litter Incidence	N(X)	0	2(9.1)	1(4.2)	2(8.3)
Fetal Incidence	N(X)	0	3(1.6) ^{e,f,g}	1(0.5) ⁿ	3(1.4) ^{r,s,u}
Wavy					
Litter Incidence	N(X)	0	4(18.2)	1(4.2)	3(12.5)
Fetal Incidence	N(X)	0	6(3.2) ^{e,f,g}	2(1.0) ^{m,n}	8(3.9) ^{r,s,t,u,v}
			**		**

PROTOCOL 120-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 12 (PAGE 2): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	182	190	195	207
Live Fetuses	N	182	190	195	207
Dead Fetuses	N	0	0	0	0

RIBS (CONTINUED):

Cervical Rib at the 7th Cervical Vertebra

Litter Incidence	N(2)	0	0	1(4.2)	0
Fetal Incidence	N(2)	0	0	1(0.5)	0

STERNEBRAE SUMMARIZATION (Includes incompletely or unossified sternebrae):

Litter Incidence	N(2)	6(27.3)	2(9.1)	12(50.0)*	9(37.5)
Fetal Incidence	N(2)	8(4.4)	3(1.6)	13(6.7)	18(8.7)*

STERNEBRAE:

Incompletely Ossified

Litter Incidence	N(2)	5(22.7)	1(4.5)	9(37.5)	7(29.2)
Fetal Incidence	N(2)	5(2.7) ^d	2(1.0)	10(5.1) ^{1, j, k, 1}	9(4.3) ^c

Not Ossified

Litter Incidence	N(2)	2(9.1)	1(4.5)	3(12.5)	5(20.8)
Fetal Incidence	N(2)	3(1.6) ^b	1(0.5)	3(1.5) ^h	9(4.3) ^{o, p, v, w}

Fused

Litter Incidence	N(2)	0	0	0	1(4.2)
Fetal Incidence	N(2)	0	0	0	1(0.5)

Asymmetric

Litter Incidence	N(2)	0	0	1(4.2)	0
Fetal Incidence	N(2)	0	0	1(0.5) ^h	0

PROTOCOL 320-003: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 12 (PAGE 3): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	90
Litters Evaluated	N	22	22	24	24
Fetuses Evaluated	N	182	190	195	207
Live Fetuses	N	182	190	195	207
Dead Fetuses	N	0	0	0	0

SCAPULAE:

Bent

Litter Incidence	N(I)	0	0	0	1(4.2)
Fetal Incidence	N(I)	0	0	0	1(0.5) ^f

PELVIS SUMMARIZATION (Includes incompletely or unossified pubes and ischia):

Litter Incidence	N(I)	4(18.2)	1(4.5)	6(25.0)	4(16.7)
Fetal Incidence	N(I)	5(2.7)	1(0.5)	10(5.1)	9(4.3)

PELVIS:

Pubes, Incompletely Ossified

Litter Incidence	N(I)	3(13.6)	0	6(25.0)	4(16.7)
Fetal Incidence	N(I)	4(2.2) ^{b,d}	0	10(5.1) ^{l,j,k, l.m,n}	7(3.4) ^{o,q,u,v}

Ischia, Incompletely Ossified

Litter Incidence	N(I)	1(4.5)	1(4.5)	1(4.2)	4(16.7)
Fetal Incidence	N(I)	1(0.5) ^d	1(0.5) ^f	1(0.5) ^l	5(2.4) ^{o,q,u,v}

Pubes, Not Ossified

Litter Incidence	N(I)	1(4.5)	0	0	1(4.2)
Fetal Incidence	N(I)	1(0.5) ^c	0	0	1(0.5) ^p

Ischia, Not Ossified

Litter Incidence	N(I)	1(4.5)	0	0	0
Fetal Incidence	N(I)	1(0.5) ^{c440}	0	0	0

PROTOCOL 320-005: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCYL ESTER) ADMINISTERED ORALLY VIA GAVAGE TO Crl:CD(TM)BR VAF/Plus(TM) PRESUMED PREGNANT RATS

TABLE 12 (PAGE 4): FETAL SKELETAL ALTERATIONS - SUMMARY

FOOTNOTES

- a. Dosage occurred on days 6 through 15 of gestation.
- b. Fetus 18929-18 also had other skeletal alterations.
- c. Fetus 18937-12 also had other skeletal alterations.
- d. Fetus 18949-1 also had other skeletal alterations.
- e. Fetus 18951-11 also had other skeletal alterations.
- f. Fetus 18971-4 also had other skeletal alterations.
- g. Fetus 18971-8 also had other skeletal alterations.
- h. Fetus 18976-1 also had other skeletal alterations.
- i. Fetus 18982-6 also had other skeletal alterations.
- j. Fetus 18990-8 also had other skeletal alterations.
- k. Fetus 18992-13 also had other skeletal alterations.
- l. Fetus 18994-14 also had other skeletal alterations.
- m. Fetus 19000-1 also had other skeletal alterations.
- n. Fetus 19000-16 also had other skeletal alterations.
- o. Fetus 19005-13 also had other skeletal alterations.
- p. Fetus 19005-15 also had other skeletal alterations.
- q. Fetus 19011-1 also had other skeletal alterations.
- r. Fetus 19014-1 also had other skeletal alterations.
- s. Fetus 19014-6 also had other skeletal alterations.
- t. Fetus 19014-10 also had other skeletal alterations.
- u. Fetus 19017-7 also had other skeletal alterations.
- v. Fetus 19025-5 also had other skeletal alterations.

* Significantly different from the vehicle control group value ($P \leq 0.05$).

** Significantly different from the vehicle control group value ($P \leq 0.01$).

PRIMARY REVIEWER: Jes. Rowland, Toxicologist *Jim Rowland 8/14/92*
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head *K. Clark Swentzel 8/19/92*
Section II, Toxicology Branch II

DATA EVALUATION REPORT

3. RANGE-FINDING STUDY--RABBIT

STUDY TYPE: Developmental Toxicity [Range-Finding] **GUIDELINE:** N/A

CASWELL NO. 315 AS **MRID No.** 423046-04 **DP Barcode:** D179762

TEST MATERIAL: 2-Ethylhexyl Ester of 2,4-D ACID [2,4-D-EHE]

REGISTRANT: Industry Task Force on 2,4-D Research Data.

TESTING LABORATORY: Argus Research Laboratories, PA

STUDY IDENTIFICATION: Argus 320-006P

TITLE OF REPORT: DOSAGE-RANGE DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOOCTYL ESTER] ADMINISTERED ORALLY [STOMACH TUBE] TO NEW ZEALAND WHITE RABBITS.

AUTHOR: Terry Martin, D.V.M **REPORT DATE:** April 10, 1992

SUMMARY: In a dose range-finding study, impregnated New Zealand White rabbits were given oral administration of Technical 2,4-D-2-ethylhexyl ester [63.25% acid equivalent; 95% pure] in aqueous methylcellulose [1%] at acid equivalent doses of 0, 50, 100, 150 or 200 mg/kg/day during days 6 through 18 of gestation. Does were sacrificed on gestation day 29. No maternal toxicity was seen at 50 mg/kg/day. 2,4-D-2-EHE induced maternal toxicity which included mortality/morbidity, clinical signs of toxicity, decreases in body weight gain and food consumption, and gross pathological alterations at 100, 150 and 200 mg/kg/day. Treatment had no adverse effects on pregnancy rate, the number of implantations, the number of resorptions, litter size, or viability of fetuses at 50 mg/kg/day. However, maternal toxicity at higher dose levels precluded meaningful evaluation of these parameters at higher levels. No fetal data were reported.

MATERNAL TOXICITY NOEL = 50 mg/kg/day [LDT]; **LOEL** = 100 mg/kg/day

DEVELOPMENTAL TOXICITY NOEL = 50 mg/kg/day; **LOEL** = 100 mg/kg/day.

CORE CLASSIFICATION: Not applicable; range-finding study.

1. **OBJECTIVE**

The objective of this range-finding study in rabbits was to establish appropriate dose levels of the 2-ethylhexyl ester of 2,4-D [2,4-D-2-EHE] for the main study.

2. **PROTOCOL**

Groups of five inseminated female New Zealand White rabbits [Hra:(NZW)SPF] approximately 5 months of age and weighing 2.5 to 5.5 kg were given oral administrations of Technical 2,4-D-2-EHE [62,25% acid equivalent, 95% pure, Lot No.04KF54479] at acid equivalent doses of 50, 100, 150, or 200 mg/kg/day in 1% methylcellulose [10 mL/kg] daily, during days 6 through 18 of gestation. A control group consisting of four rabbits received the vehicle alone under the same schedule. Concentration and homogeneity of the test article/vehicle mixtures were determined prior to the initiation of the study.

Animals were observed daily for viability, clinical signs of toxicity, abortions, premature deliveries, body weights and food consumptions during the dosing and post-dosing periods. Dams in each group were sacrificed on day 29 and postmortem examination included macroscopic examination of internal organs, with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. Fetal examinations were not performed.

3. RESULTS

i. Analysis of dosing solution

The mean concentrations found were 82%, 94%, 76% and 96% of the nominal concentration, respectively on the first day of dosing, and 96%, 87%, 75%, and 84% on the last day of dosing, respectively, for the 50, 100 and 150 mg/kg/day groups. Dosing solution exhibited 100% homogeneity.

ii. Maternal Toxicity

- o 50 mg/kg/day: No mortality
- o 100 mg/kg/day: 40% [2/5] mortality; 1 doe found dead on day 21 & 1 doe sacrificed moribund on Day 13.
- o 150 mg/kg/day: 60% [3/5] mortality; 1 doe each found dead on Days 17 & 18 and 1 doe sacrificed moribund on Day 9.
- o 200 mg/kg/day: 100% [5/5] mortality 1 doe each found dead on Days 9 & 11; 2 does found dead on Day 12 and 1 doe sacrificed on Day 10.
- o Because of deaths/moribund sacrifices, the 100 and 150 mg/kg/day groups were not continued on study after Day 21 and 19, respectively.
- o Treatment-related clinical signs of toxicity observed in does [at 100, 150 and 200 mg/kg/day] prior to death or sacrifice included hyperactivity, myotonia, decreased motor activity, lost righting reflex, impaired righting reflex, labored breathing and/or ataxia. No treatment-related clinical signs were seen at 50 mg/kg/day.
- o Body weight gain was unaffected at 50 mg/kg/day. Body weight loss occurred at 100 mg/kg/day [Days 6 to 9 & Days 15 to 19], 150 mg/kg/day [Days 12 to 15 and Days 15 to 19], and 200 mg/kg/day [Days 6 to 9]. However, deaths and moribund sacrifices at these higher levels precluded further meaningful evaluation of body weights or body weight gains.

- o Food consumption was unaffected at 50 mg/kg/day. Decreases in food consumption occurred in the 100 mg/kg/day [Days 6 to 9; 9 to 12; 15 to 19; 6-19], 150 mg/kg/day [Days 12 to 15 & Days 15 to 19], and 200 mg/kg/day [Days 6 to 9]. However, deaths and moribund sacrifices at these higher levels precluded further meaningful evaluation.
- o Treatment-related gross pathological alterations seen in rabbits found dead or sacrificed moribund were a red substance in the anogenital area in one doe at 100 mg/kg/day; ulcerations in the gastric fundus in one doe at 150 and in another doe at 200 mg/kg/day; and distended urinary bladder in one doe at 100 mg/kg/day and two does at 200 mg/kg/day. All other necropsy observations were not considered to be treatment related.
- o The pregnancy rate was 100%, 100%, 80%, 100% and 80% at 0, 50, 100, 150 and 200 mg/kg/day dose groups, respectively.

iii. Developmental Toxicity

- o Since there were no surviving dams at 100, 150 or 200 mg/kg/day at termination, cesarean section data is based on the 50 mg/kg/day.
- o No marked treatment-related effects were observed in implantations, litter sizes, or viable fetuses at 50 mg/kg/day when compared to controls. The average number of early and late resorptions and the number of does with any resorptions at 50 mg/kg/day were slightly higher than the concurrent control group values but were within the range of historical control values. No doe had a litter of only resorbed conceptuses, and there were no dead fetuses. Maternal deaths or sacrifices precluded examination of these parameters in does given higher dose levels.
- o No data were reported for fetal examinations.

4. CONCLUSION:

MATERNAL TOXICITY: NOEL = 50 mg/kg/day [LDT]
LOEL = 100 mg/kg/day

DEVELOPMENTAL TOXICITY: NOEL = 50 mg/kg/day [LDT]
LOEL = 100 mg/kg/day

PRIMARY REVIEWER: Jess Rowland, Toxicologist *John Rowland 8/14/92*
Section II, Toxicology Branch II

SECONDARY REVIEWER: K. Clark Swentzel, Section Head *K. Clark Swentzel*
Section II, Toxicology Branch II *8/19/92*

DATA EVALUATION REPORT

4. MAIN STUDY--RABBITS

STUDY TYPE: Developmental Toxicity [Main Study] **GUIDELINE:** 83-3(b)

CASWELL NO. 315 AS **MRID No.** 423036-03 **DP Barcode:** 179762

TEST MATERIAL: 2-Ethylhexyl Ester of 2,4-D [2,4-D-EHE]

REGISTRANT: Industry Task Force II on 2,4-D Research Data

STUDY IDENTIFICATION: Argus 320-006

TESTING LABORATORY: Argus Research Laboratories, PA.

TITLE OF REPORT: DEVELOPMENTAL TOXICITY [EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL] STUDY OF 2,4-D 2-ETHYLHEXYL ESTER [2,4-D ISOCTYL ESTER] ADMINISTERED ORALLY [STOMACH TUBE] TO NEW ZEALAND WHITE RABBITS.

AUTHOR: Terry Martin, D.V.M **REPORT DATE:** April 10, 1992

SUMMARY: Groups of 20 inseminated rabbits were given oral administration of 2-ethylhexyl ester of 2,4-D [2,4-D-2EHE] at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 18 of gestation. 2,4-D-2-EHE at 10 or 30 mg/kg/day did not induce maternal toxicity. At 75 mg/kg/day maternal toxicity was manifested by mortality/morbidity, decreases in mean body weight, body weight gain and food consumption in does that died or sacrificed moribund, and clinical signs of toxicity characterized by dried feces, decreased motor activity, ataxia, impaired righting reflex, lost righting reflex and bradypnea. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external, visceral, or skeletal malformation or variations were seen in any of the fetuses of treated does. Based on these results the following NOELs and LOELs are established.

MATERNAL TOXICITY: NOEL = 30 mg/kg/day; LOEL = 75 mg/kg/day [NMT]

DEVELOPMENTAL TOXICITY: NOEL = 75 mg/kg/day; LOEL = Not Achieved

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

I. OBJECTIVE

The objective of this study was to assess the effects of the 2-Ethylhexyl ester of 2,4-D [2,4-D-EHE] on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: 2-ethylhexyl ester of 2,4-D [Technical]
Batch No.: 04KF54479
Acid Equivalent: 63.25%
Purity: 95%
Description: Yellow liquid

b. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White [Hra: (NZW) SPF]
Age at receipt: Approximately 6 months
Weight at randomization: 3.11 - 4.02 kg
Identification: Ear tags.
Acclimation Period: 28 days.
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322 ad libitum.
Water: Tap water ad libitum
Environment: Temperature-60-70°F; humidity-40-70%; light cycle: 12 hr. light/12 hr. dark

Group Assignment: 20 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Adult females were artificially inseminated with the day of insemination considered Day 0 of gestation. Does were given intravenous injection of 20 USP Units/kg of human chorionic gonadotropin approximately three hours prior to insemination. An estimated 0.25 mL of semen that had been diluted with normal saline to a concentration of 6.0×10^6 spermatozoa/0.25 mL saline was used for insemination.

d. Preparation of Dosing Solutions

Suspensions of 2,4-D-EHE in aqueous 1% methylcellulose were prepared daily. Dosage calculations were corrected for the 63.25% acid equivalence of the test substance and expressed as mg of the acid.

e. Analysis of the Dosing Solutions

Concentration analysis of the dosing solutions were determined twice [on the first and the last days] during the study. Homogeneity was determined prior to the beginning of the dosage period. Since dosing samples were prepared daily, no additional stability analyses were performed.

f. Administration of Test Article

The test article was administered daily orally via gavage at doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 18 of gestation. All groups received a dosing volume of 10 mL/kg body weight and the dose volumes were adjusted daily based on individual body weights. Each daily dosage given to the rabbits was administered at approximately the same time each day.

g. Observations

All animals were observed for clinical signs of toxicity, abortions, premature deliveries and deaths immediately prior to dosing, and approximately 60 minutes post-dosing. Individual body weights were obtained on day 0 and daily during the dosing and post-dosing period. Individual food consumptions were measured daily during the study period.

h. Termination

Any animal which died, appeared moribund or showed indications of early termination of pregnancy was submitted for complete necropsy. All surviving does were sacrificed on gestation day 29, obvious gross pathologic alterations were recorded, and the gravid uteri were weighed.

i. Cesarean Section

The thoracic, abdominal and pelvic cavities were examined for gross lesions, and in the event of gross lesions, the tissues were preserved in neutral buffered 10% formalin. The uterus was removed from the body, examined externally, weighed and then opened for internal examination. Uteri that appeared to be from nonpregnant rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantations, early and late resorptions, and live and dead fetuses were recorded.

j. Fetal Examinations

Each fetus was removed from the uterus and individually weighed, and observed for gross external alterations. Every fetus was examined to determine sex and soft tissue alterations. Fetuses were then eviscerated, stained with Alizarin red-S, and examined for skeletal alterations.

k. Statistical Analysis

Maternal body weights, body weight gains, gravid uterine weights, feed consumption data, and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, anomaly average data and ossification site data were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance [ANOVA]. If the ANOVA was significant, analysis by Dunnett's Test was used. If the ANOVA was not significant, the Kruskal-Wallis Test or Fisher's Exact Test was used. All other cesarean-sectioning data were evaluated using the Kruskal-Wallis Test. Observations for aborted conceptuses and late resorptions were excluded from statistical analyses.

l. Compliance Statements:

A signed Statement of No Confidentiality Claim was provided dated April 10, 1992.

A signed Statement of Compliance with EPA's GLP was provided that was dated April 10, 1992.

A signed Quality Assurance Statement was dated April 10, 1992. This date conforms to the review of the study phases and the draft and the final reports.

A signed statement dated April 10, 1992 was provided, which indicated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the study. This study neither reportedly met or exceeded any of these criteria.

III. RESULTS

Analysis of the Dosing Solutions

Results of the concentration analyses of the dosing solutions are tabulated below:

Target Level		% Deviation from Target Dosage	
mg/mL	mg/kg/day	First Day Sample	Last Day Sample
1.0	10	-2	-7
3.0	30	-6	2
7.5	75	-11	4

Results of the homogeneity analyses showed a relative standard deviation of 8% for the 1.0 mg/mL level, 4% for the 5.0 mg/mL level, and 5% for the 20.0 mg/mL.

1. Maternal Toxicity

a. Mortality & Pregnancy Status

Dose Level [mg/kg/day]	0	10	30	75
No. on Test	20	20	20	20
Aborted	0	0	0	1
Delivered Early	0	0	0	0
Moribund Sacrifice	0	0	0	2
Found Dead	0	0	1	0

No maternal mortality/morbidity or abortions occurred at 10 mg/kg/day.

At 30 mg/kg/day, the doe that died on Day 21 did not exhibit any adverse clinical signs of toxicity however, did show body weight loss and reduced food consumption. Necropsy revealed a red substance in the perianal area that may have been interrelated with resorption of the litter [the litter of this doe had 9 late resorption].

At 75 mg/kg/day, the doe that aborted on Day 23 showed clinical signs, body weight loss, and decreased food consumption. No gross lesions were seen at necropsy. The litter of this doe had one early and six late resorptions. The one doe that was sacrificed moribund on Day 15 exhibited clinical signs, body weight loss, reduced food consumption, and red cardiac and fundic regions in the stomach and parovarian cysts. The litter of this doe had 10 early resorptions. The doe that was sacrificed on Day 16 exhibited body weight loss, reduced food consumption, clinical signs, distended bladder with red brown fluid, clear, red fluid in the thoracic cavity, brown spots in all lung lobes and parovarian cysts. The litter of this doe had eight early resorptions.

b. Clinical Signs

No clinical signs of toxicity were seen at 0, 10 or 30 mg/kg/day. Treatment-related clinical signs of toxicity observed at 75 mg/kg/day included dried feces, decreased motor activity, ataxia, impaired righting reflex, lost righting reflex and bradypnea.

c. Body Weight Changes

As shown below, no statistically or biologically significant differences were seen in mean body weight or body weight gains between the control and treated groups during the pre-dosing period [Days 0 to 6], the dosing period [Days 6 to 19], the post-dosing period [Days 19 to 29], and the entire study [Days 0 to 29].

Dose mg/kg/day	Mean Body weight Gain [G]			
	Days 0 - 6	Days 6 - 19	Days 19 - 29	Days 0 - 29
0	+0.18±0.06	+0.21±0.10	+0.11±0.14	+0.03±0.17
10	+0.18±0.06	+0.18±0.06	+0.13±0.09	+0.03±0.15
30	+0.19±0.04	+0.20±0.03	+0.16±0.10	+0.09±0.19
75	+0.18±0.06	+0.17±0.16	+0.14±0.12	+0.09±0.14

d. Food Consumption

No treatment-related or statistically significant differences were observed in absolute [g/day] or relative [g/kg/day] food consumption values between treated and control groups during the pre-dosing, dosing, post-dosing or the entire study periods.

e. Macroscopical Examination

No treatment-related macroscopical changes were observed in the dams sacrificed at termination. Gross pathological alterations observed in the does that died or were sacrificed moribund are discussed under Mortality [see Page].

2. Developmental Toxicity

Reproduction data are presented in Table 1. No biologically or statistically significant effects were seen on pregnancy rate, number of implantations, total live fetuses per litter, resorption rate, number and percent of litters with resorptions, fetal sex ratio, fetal body weights, or gravid uterine weights at any dose level. The incidences were reported to be within the historical control ranges of the testing laboratory. This was substantiated with historical control data from the testing laboratory.

Table 1. Cesarean Section Observations

Observations [Mean \pm S.D.]	Dose Level (mg/kg/day)			
	0	10	30	75
No. Assigned	20	20	20	20
Females Gravid	18	16	16	20
<u>Maternal Wastage</u>				
# Died	0	0	0	0
# Sacrificed	0	0	0	2
# Aborted	0	0	1	0
# Early delivery	0	0	0	0
# Non pregnant	2	4	4	0
Total Corpora Lutea	183	159	151	181
Corpora Lutea/Dam	10.2 \pm 2.8	9.9 \pm 1.5	10.1 \pm 2.4	10.6 \pm 2.1
Total Implantations	131	133	118	112
Implantations/Dam	7.3 \pm 2.3	8.3 \pm 1.8	7.9 \pm 2.6	6.6 \pm 2.4
Total Live Fetuses	128	131	114	110
Live Fetuses/Litter	6.6 \pm 2.2	6.6 \pm 2.2	7.6 \pm 3.3	7.1 \pm 1.4
Total Resorptions	2	2	4	2
Early	2	2	4	2
Late	0	0	0	0
Resorption/Dam	0.2 \pm 0.4	0.1 \pm 0.3	0.3 \pm 0.4	0.1 \pm 0.5
No. and % of Litters with Resorptions	3/18 16.7	2/16 12.5	4/16 25.0	1/20 5.0
Pre Implantation Loss [%]	28.4	16.4	21.9	38.1
Post Implantation Loss [%]	2.3	1.5	3.4	1.8
Gravid Uterus Weight [g]	456 \pm 101	460 \pm 127	483 \pm 159	421 \pm 119
Sex Ratio σ / φ	63/65	62/69	64/50	61/49
Fetal Weight [g]	47 \pm 7	48 \pm 18	57 \pm 17	59 \pm 18

As summarized in Table 2, there were no treatment-related or statistically significant differences in fetal alterations in the number of litters with fetuses with any alterations noted, the number of fetuses with any alterations noted or the average percent of fetuses with any alterations per litter.

Table 2. Summary of Fetal Alterations

Observations	Dose Level [mg/kg/day]			
	0	10	30	75
Litters Evaluated	18	16	14	17
Fetuses Evaluated	128	131	114	110
Litters with Fetuses with any Alterations Observed	17 94.4%	15 93.8%	14 100%	16 94.1%
Fetuses with any Alterations Observed	59 46.1%	58 44.3%	53 46.5%	51 46.4%
Percentage of Fetuses with any Alterations/Litter [Mean±S.D]	47 ± 25	46 ± 24	45 ± 20	50 ± 30

The fetal malformations/alterations observed in the 128, 131, 114 and 110 fetuses of does at 0, 10, 30, and 75 mg/kg/day and summarized in Tables 11 thru 14 of the study report [pages 57-6] are appended to this DER.

a. External Examinations

No treatment-related or statistically significant gross external malformations or variations were seen.

b. Visceral Examinations

No treatment-related or statistically significant soft tissue malformations were observed. Statistically significant soft tissue variations included agenesis at 10 and 30 mg/kg/day and alterations in brain at 75 mg/kg/day.

Statistically significant increased incidences of agenesis of the intermediate lobe of the lungs were seen in 5 of 131 fetuses [3.8%] in two litters at 10 mg/kg/day [$p < 0.01$] and in 3 of 114 fetuses [2.6%] of in two litters at 30 mg/kg/day [$p < 0.05$]. Although these incidences showed statistical significance at the fetal incidence, they were not considered to be treatment-related since: (i) the litter incidence [12.5% at 10 mg/kg/day and 14.3% at 30 mg/kg/day] was not significantly increased; (ii) this anomaly was not seen at the high dose; and (iii) both the fetal [0-5.15] and the litter [0-31%] incidences were within the historical control range of the testing laboratory.

Moderate dilation of the lateral ventricles of the brain was observed in 4 of 110 fetuses [3.6%] at 75 mg/kg/day compared to none in the vehicle controls [$p < 0.01$]. Increase in this variation was not considered to be treatment-related since it occurred in only one high dose group litter, the litter incidence [1/17, 5.9%] was not statistically significant, and both the fetal [0-2.5%] and litter [0-17%] incidences were within the historical control range of the testing laboratory.

c. Skeletal Examinations

No other treatment-related or statistically significant skeletal malformations were seen between the control and the treated groups.

IV. DISCUSSION

Inseminated rabbits were given oral administration of 2-ethylhexyl ester of 2,4-D at acid equivalent doses of 0, 10, 30, or 75 mg/kg/day during days 6 through 18 of gestation.

2,4-D-2-EHE at 10 or 30 mg/kg/day did not induce maternal toxicity. At 75 mg/kg/day maternal toxicity was manifested by mortality/morbidity, decreases in mean body weight, body weight gain and food consumption in does that died or sacrificed moribund, and clinical signs of toxicity characterized by dried feces, decreased motor activity, ataxia, impaired righting reflex, lost righting reflex and bradypnea. No treatment-related effects were observed in reproductive parameters at any dose level. No treatment-related external, visceral, or skeletal malformation or variations were seen in any of the fetuses of treated does.

In a developmental toxicity study [MRID No. 423046-01] with rats, pregnant Sprague-Dawley rats were given oral administration of 2,4-D-2-EHE [same lot number and purity [98%] as the rabbit study] at acid equivalent doses of 0, 10, 30, or 90 mg/kg/day during days 6 - 15 of gestation. For maternal toxicity the NOEL was 10 mg/kg/day and the LOEL, based on decreases in body weight gain and food consumption, was 30 mg/kg/day. For developmental toxicity the NOEL was 10 mg/kg/day and the LOEL, based on delayed sternal ossification, was 30 mg/kg/day. The lack of any developmental toxicity in rabbits compared to skeletal variations in rats at comparable acid equivalent doses [75 mg/kg/day in rabbits vs 90 mg/kg/day in rats] indicate the species difference in 2,4-D-EHE induced developmental toxicity.

V. CONCLUSION

2,4-D-2-EHE was maternally toxic to rabbits at a dose of 75 mg/kg/day; no maternal toxicity was seen at 10 or 30 mg/kg/day. The compound did not induce developmental toxicity at 75 mg/kg/day, the highest dose tested. Based on the results of this study, the following NOELs and LOELs are established.

Maternal Toxicity

NOEL: 30 mg/kg/day

LOEL: 75 mg/kg/day [HDT]

Developmental Toxicity:

NOEL: 95 mg/kg/day [HDT]

LOEL: Not Achieved

VI. CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3 b) and is acceptable for regulatory purposes.

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TABLE 11 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	75
Litters Evaluated	N	18 ^b	16	14	17
Fetuses Evaluated	N	128 ^b	131	114	110
Live Fetuses	N	128 ^b	131	114	110
Dead Fetuses	N	0	0	0	0

HEAD:

Encephalocele

Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0

BODY:

Meningomyelocele (Lumbar Region)

Litter Incidence	N(%)	0	0	0	1(5.9)
Fetal Incidence	N(%)	0	0	0	1(0.9)

Subcutaneous Hemorrhage (Whole Body)

Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9) ^c	0

FORELIMBS:

Bilateral, Paws, Flexed

Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9) ^c	0

- a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D 2-Ethylhexyl Ester.
- b. Includes values for litter 19768, in which gross external examination was performed to the extent possible because the heads of these fetuses were skinned prior to examination.
- c. Fetus 19799-4 also had other gross external alterations.

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TABLE 12 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0

BRAIN:

Small

Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0

Hist

Lateral Ventricles, Moderate Dilation

Litter Incidence	N(%)	0	0	0	1(5.9)
Fetal Incidence	N(%)	0	0	0	4(3.6)**

LUNGS:

Intermediate Lobe, Agenesis

Litter Incidence	N(%)	0	2(12.5)	2(14.3)	0
Fetal Incidence	N(%)	0	5(3.8)**	3(2.6) ^{b*}	0

GALLBLADDER:

Absent

Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9) ^b	0

KIDNEY:

Left, Pelvis, Marked Dilation

Litter Incidence	N(%)	1(5.6)	0	0	0
Fetal Incidence	N(%)	1(0.8)	0	0	0

- a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D 2-Ethylhexyl Ester.
- b. Fetus 19799-4 also had other soft tissue alterations.
- * Significantly different from the vehicle control group value ($P \leq 0.05$).
- ** Significantly different from the vehicle control group value ($P \leq 0.01$).

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TABLE 13 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0

SKULL - IRREGULAR OSSIFICATION^b:

(SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF SKULL^c; SUMMARIZED AND INDIVIDUAL SUBCATEGORIES CITED BELOW)

Litter Incidence	N(I)	16(88.9)	15(93.8)	14(100.0)	15(88.2)
Fetal Incidence	N(I)	53(41.4)	52(39.7)	50(43.8)	48(43.6)

NASAL(S), IRREGULAR OSSIFICATION

(SUMMARIZATION OF INTERNASALS; INTRANASALS; IRREGULAR SUTURE; MIDLINE SUTURE DISPLACED; NASAL(S)-FRONTAL(S), IRREGULAR SUTURE)

Litter Incidence	N(I)	15(83.3)	15(93.8)	12(85.7)	14(82.4)
Fetal Incidence	N(I)	35(27.3)	41(31.3)	33(28.9)	29(26.4)

Nasals, Internasal

Litter Incidence	N(I)	1(5.6)	3(18.8)	0	2(11.8)
Fetal Incidence	N(I)	1(0.8)	3(2.3)	0	2(1.8)

Nasal(s), Intranasal

Litter Incidence	N(I)	1(5.6)	1(6.2)	0	2(11.8)
Fetal Incidence	N(I)	1(0.8)	3(2.3)	0	2(1.8)

Nasals, Irregular Suture

Litter Incidence	N(I)	1(5.6)	2(12.5)	2(14.3)	2(11.8)
Fetal Incidence	N(I)	1(0.8)	2(1.5)	2(1.8)	2(1.8)

Nasals, Midline Suture Displaced

Litter Incidence	N(I)	15(83.3)	14(87.5)	12(85.7)	13(76.5)
Fetal Incidence	N(I)	27(21.1) ^d	28(21.4)	29(25.4) ^{e,h}	21(19.1) ^{j,l}

Nasal(s)-Frontal(s), Irregular Suture

Litter Incidence	N(I)	5(27.8)	5(31.2)	3(21.4)	2(11.8)
Fetal Incidence	N(I)	7(5.5)	11(8.4)	4(3.5)	6(5.4)

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TABLE 13 (PAGE 2): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0

SKULL - IRREGULAR OSSIFICATION (CONTINUED)^b:

FRONTAL(S), IRREGULAR OSSIFICATION

(SUMMARIZATION OF INTERFRONTALS; INTRAFRONTALS; IRREGULAR SUTURE; SUTURE ENLARGED)

Litter Incidence	N(Z)	12(66.7)	12(75.0)	13(92.8)	12(70.6)
Fetal Incidence	N(Z)	25(19.5)	19(14.5)	25(21.9)	27(24.5)

Frontals, Interfrontal

Litter Incidence	N(Z)	9(50.0)	6(37.5)	7(50.0)	6(35.3)
Fetal Incidence	N(Z)	11(8.6) ^c	7(5.3)	9(7.9)	9(8.2) ^k

Frontal(s), Intrafrontal

Litter Incidence	N(Z)	4(22.2)	2(12.5)	1(7.1)	2(11.8)
Fetal Incidence	N(Z)	4(3.1)	2(1.5)	1(0.9)	2(1.8)

Frontals, Irregular Suture

Litter Incidence	N(Z)	7(38.9)	8(50.0)	10(71.4)	9(52.9)
Fetal Incidence	N(Z)	12(9.4)	9(6.9)	16(14.0) ^{8, i}	18(16.4)

Frontals, Suture Enlarged

Litter Incidence	N(Z)	0	1(6.2)	0	0
Fetal Incidence	N(Z)	0	1(0.8)	0	0

PARIETAL(S), IRREGULAR OSSIFICATION

(SUMMARIZATION OF INTRAPARIETAL; SUTURE ENLARGED)

Litter Incidence	N(Z)	1(5.6)	1(6.2)	0	0
Fetal Incidence	N(Z)	1(0.8)	1(0.8)	0	0

Parietal, Intraparietal

Litter Incidence	N(Z)	1(5.6)	0	0	0
Fetal Incidence	N(Z)	1(0.8)	0	0	0

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TABLE 13 (PAGE 3): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0

SKULL - IRREGULAR OSSIFICATION (CONTINUED)^b:

Parietals, Suture Enlarged

Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0

SUPRAOCCIPITALS, IRREGULARLY SHAPED

Litter Incidence	N(%)	0	1(6.2)	0	0
Fetal Incidence	N(%)	0	1(0.8)	0	0

HYOID^b:

Ala(e), Angulated

Litter Incidence	N(%)	2(11.1)	1(6.2)	3(21.4)	3(17.6)
Fetal Incidence	N(%)	3(2.3)	1(0.8)	3(2.6)	3(2.7) ^k

Ala(e), Short

Litter Incidence	N(%)	1(5.6)	1(6.2)	0	0
Fetal Incidence	N(%)	2(1.6)	1(0.8)	0	0

VERTEBRAE:

Cervical, Hemivertebra

Litter Incidence	N(%)	0	0	1(7.1)	1(5.9)
Fetal Incidence	N(%)	0	0	1(0.9) ^h	1(0.9) ^l

Cervical, Centrum, Unilateral Ossification

Litter Incidence	N(%)	0	0	1(7.1)	0
Fetal Incidence	N(%)	0	0	1(0.9) ^h	0

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TABLE 13 (PAGE 4): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0

VERTEBRAE (CONTINUED):

Cervical, Centra, Fused

Litter Incidence	N(X)	0	0	1(7.1)	0
Fetal Incidence	N(X)	0	0	1(0.9) ^h	0

Thoracic, Hemivertebra

Litter Incidence	N(X)	0	0	1(7.1)	0
Fetal Incidence	N(X)	0	0	1(0.9) ^f	0

Thoracic, Centrum, Unilateral Ossification

Litter Incidence	N(X)	0	0	1(7.1)	0
Fetal Incidence	N(X)	0	0	1(0.9) ^h	0

Thoracic, Centra, Fused

Litter Incidence	N(X)	0	0	1(7.1)	0
Fetal Incidence	N(X)	0	0	1(0.9) ^h	0

Lumbar, Centra, Fused

Litter Incidence	N(X)	0	0	1(7.1)	0
Fetal Incidence	N(X)	0	0	1(0.9) ^f	0

Lumbar, Arch, Small

Litter Incidence	N(X)	0	0	0	1(5.9)
Fetal Incidence	N(X)	0	0	0	1(0.9) ^j

Caudal, Misaligned

Litter Incidence	N(X)	1(5.6)	0	0	0
Fetal Incidence	N(X)	1(0.8) ^g	0	0	0

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TABLE 13 (PAGE 5): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	10	30	75
Litters Evaluated	N	18	16	14	17
Fetuses Evaluated	N	128	131	114	110
Live Fetuses	N	128	131	114	110
Dead Fetuses	N	0	0	0	0

RIBS:

Split

Litter Incidence	N(2)	0	0	1(7.1)	0
Fetal Incidence	N(2)	0	0	1(0.9) ^h	0

STERNEBRAE SUMMARIZATION (Includes incompletely ossified, fused and asymmetric sternebrae):

Litter Incidence	N(2)	2(11.1)	2(12.5)	2(14.3)	3(17.6)
Fetal Incidence	N(2)	2(1.6)	4(3.0)	2(1.8)	3(2.7)

STERNEBRAE:

Incompletely Ossified

Litter Incidence	N(2)	0	0	0	2(11.8)
Fetal Incidence	N(2)	0	0	0	2(1.8) ^{k,1}

Fused

Litter Incidence	N(2)	2(11.1)	2(12.5)	2(14.3)	1(5.9)
Fetal Incidence	N(2)	2(1.6) ^d	4(3.0)	2(1.8) ^{8,1}	1(0.9)

Asymmetric

Litter Incidence	N(2)	1(5.6)	0	0	0
Fetal Incidence	N(2)	1(0.8) ^d	0	0	0

009697

PROTOCOL 320-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2-ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 13 (PAGE 6): FETAL SKELETAL ALTERATIONS - SUMMARY

FOOTNOTES

- a. Dosage occurred on days 6 through 18 of gestation; all dosages are reported as the acid equivalent of 2,4-D contained in the 2,4-D 2-Ethylhexyl Ester.
- b. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summary table, except when alterations of other ossification sites were also present.
- c. Includes all findings noted for the skull except hyoid, ala(e), angulated; ala(e), short. These categories are excluded because they are not considered to result from irregular ossification.
- d. Fetus 19757-2 also had other skeletal alterations.
- e. Fetus 19770-3 also had other skeletal alterations.
- f. Fetus 19796-1 also had other skeletal alterations.
- g. Fetus 19801-8 also had other skeletal alterations.
- h. Fetus 19803-4 also had other skeletal alterations.
- i. Fetus 19803-9 also had other skeletal alterations.
- j. Fetus 19818-3 also had other skeletal alterations.
- k. Fetus 19818-4 also had other skeletal alterations.
- l. Fetus 19825-7 also had other skeletal alterations.

009607

PROTOCOL 120-006: DEVELOPMENTAL TOXICITY (EMBRYO-FETAL TOXICITY AND TERATOGENIC POTENTIAL) STUDY OF 2,4-D 2 ETHYLHEXYL ESTER (2,4-D ISOOCTYL ESTER) ADMINISTERED ORALLY VIA STOMACH TUBE TO NEW ZEALAND WHITE RABBITS

TABLE 14 (PAGE 1): FURAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSE GROUP		I	II	III	IV
DOSE (MG/ML/DAY) ^a		0 (VEHICLE)	10	30	75
LITTERS EXAMINED	N	18	16	14	17
FETUSES EXAMINED	N	128	131	114	110
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN ± S.D.	0.99 ± 0.02	1.00 ± 0.00	1.00 ± 0.00	0.99 ± 0.05
VERTEBRAE					
CERVICAL	MEAN ± S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN ± S.D.	12.66 ± 0.20	12.54 ± 0.31	12.72 ± 0.32	12.60 ± 0.30
LUMBAR	MEAN ± S.D.	6.34 ± 0.29	6.44 ± 0.31	6.26 ± 0.30	6.31 ± 0.31
SACRAL	MEAN ± S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN ± S.D.	17.14 ± 0.49	16.81 ± 0.44	17.07 ± 0.27	17.19 ± 0.40
RIBS (PAIRS)	MEAN ± S.D.	12.57 ± 0.29	12.51 ± 0.30	12.61 ± 0.28	12.64 ± 0.30
STERNUM					
MANUBRIUM	MEAN ± S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CARTILAGE	MEAN ± S.D.	3.99 ± 0.02	3.97 ± 0.10	3.98 ± 0.04	3.96 ± 0.12
XIPHOID	MEAN ± S.D.	0.09 ± 0.19	0.92 ± 0.25	0.91 ± 0.18	0.06 ± 0.18
PHALANX b					
CARPALS	MEAN ± S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN ± S.D.	4.99 ± 0.02	4.99 ± 0.05	4.99 ± 0.03	5.00 ± 0.00
DIGITS	MEAN ± S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANXES	MEAN ± S.D.	13.94 ± 0.09	13.94 ± 0.09	13.95 ± 0.09	13.07 ± 0.31
PHALANX b					
TARSALS	MEAN ± S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
METATARSALS	MEAN ± S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN ± S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANXES	MEAN ± S.D.	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	11.99 ± 0.05

a. Doseage occurred on days 6 through 10 of gestation; all doseages are reported as the acid equivalent of 2,4-D contained in the 2,4-D 2-Ethylhexyl Ester.

b. Calculated as average per limb.

END